Reply to Office Action Dated: May 11, 2009

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

LISTING OF CLAIMS:

1. (Currently Amended): A process for the production of L-ascorbic acid

comprising:

contacting an enzyme with a substrate which is selected from the (a)

group consisting of L-gulose, L-galactose, L-idose, and L-talose;

converting the substrate directly into L-ascorbic acid by catalytic (b)

activity of the enzyme in a microorganism capable of such conversion at a pH of about 1

to about 9 and at a temperature of about 13°C to about 45°C under suitable culture

conditions; and

(c) isolating L-ascorbic acid from the reaction mixture.

wherein said enzyme has (1) the amino acid sequence of SEQ ID NO: 2 or (2) an amino

acid sequence encoded by the DNA sequence of SEQ ID NO: 1 or (3) an amino acid

sequence encoded by a DNA sequence that hybridizes to the full-length complement of

the DNA sequence of SEQ ID NO: 1 under highly stringent hybridization conditions of

6X SSC, 0.5% SDS, 100 µg/ml denatured salmon sperm DNA, 50% formamide, and

incubating overnight at 42°C with gentle rocking and highly stringent wash conditions of

washing in 2X SSC, 0.5%SDS at room temperature for 15 minutes, followed by another

wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes and having the

activity to produce L-ascorbic acid.

3

Amendment Dated: November 10, 2009 Reply to Office Action Dated: May 11, 2009

2. (Currently Amended): A process for the production of L-ascorbic acid with an enzyme having (1) the amino acid sequence of SEQ ID NO: 2 or (2) an amino acid sequence encoded by the DNA sequence of SEQ ID NO: 1 or (3) an amino acid sequence encoded by a DNA sequence that hybridizes to the <u>full-length</u> complement of the DNA sequence of SEQ ID NO: 1 under highly stringent hybridization conditions of 6X SSC, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA, 50% formamide, and incubating overnight at 42°C with gentle rocking and highly stringent wash conditions of washing in 2X SSC, 0.5% SDS at room temperature for 15 minutes, followed by another wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes and having the activity to produce L-ascorbic acid, whereby L-ascorbic acid is produced from a substrate which is selected from the group consisting of L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone, and L-talonic acid,

said process comprising the steps of:

- (a) contacting the enzyme with the substrate,
- (b) converting the substrate directly into L-ascorbic acid by catalytic activity of the enzyme in a microorganism capable of such conversion at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C under suitable culture conditions; and
 - (c) isolating L-ascorbic acid from the reaction mixture.
- 3. (Withdrawn): A process for the production of L-gulono-1,4-lactone or L-gulonic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, with the activity to produce L-gulono-

Amendment Dated: November 10, 2009 Reply to Office Action Dated: May 11, 2009

1,4-lactone or L-gulonic acid, whereby L-gulono-1,4-lactone or L-gulonic acid is produced from L-gulose.

- 4. (Withdrawn): A process for the production of L-galactono-1,4-lactone or L-galactonic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, with the activity to produce L-galactono-1,4-lactone or L-galactonic acid, whereby L-galactono-1,4-lactone or L-galactonic acid is produced from L-galactose.
 - 5. (Cancelled).
- 6. (Currently amended): A process according to claim 1, wherein the process is conducted for 1 to 120 hours [[h]] at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.
- 7. (Original): A process according to claim 6, wherein the process is conducted at a pH of about 2 to about 8 and at a temperature of about 18°C to about 42°C.
- 8. (Currently Amended): A process for producing L-ascorbic acid comprising (a) contacting a substrate which is selected from the group consisting of L-gulose, L-galactose, L-idose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, and L-galactonic acid with an enzyme obtained derivable from G. oxydans DSM 4025, (b) converting the substrate directly into L-ascorbic acid by catalytic activity of the enzyme in a microorganism capable of such conversion at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C; under suitable culture-conditions and (c) isolating L-ascorbic acid from the reaction mixture; wherein the enzyme has the following physico-chemical properties:

Amendment Dated: November 10, 2009 Reply to Office Action Dated: May 11, 2009

- (a) molecular weight of about 60,000 Da on SDS-PAGE;
- (b) substrate specificity for primary and secondary alcohols and aldehydes;
 - (c) pH-stability at pH of about 6 to about 9;
 - (d) pH-optimum at pH of about 8.0; and
 - (e) inhibited by Cu²⁺, Zn²⁺, Mn²⁺, Fe²⁺, and Fe³⁺.
- 9. (Withdrawn): A process for producing L-gulono-1,4-lactone or L-gulonic acid comprising contacting L-gulose with Enzyme B of *G. oxydans* DSM 4025 and isolating L-gulono-1,4-lactone or L-gulonic acid from the reaction mixture, wherein Enzyme B has the following physico-chemical properties:
 - (a) molecular weight of about 60,000 Da on SDS-PAGE;
- (b) substrate specificity for primary and secondary alcohols and aldehydes;
 - (c) pH-stability at pH of about 6 to about 9;
 - (d) pH-optimum at pH of about 8.0; and
 - (e) inhibited by Cu²⁺, Zn²⁺, Mn²⁺, Fe²⁺, and Fe³⁺.
- 10. (Withdrawn): A process for producing L-galactono-1,4-lactone or galactonic acid comprising contacting L-galactose with Enzyme B of *G. oxydans* DSM 4025 and isolating L-galactono-1,4-lactone or galactonic acid from the reaction mixture, wherein Enzyme B has the following physico-chemical properties:
 - (a) molecular weight of about 60,000 Da on SDS-PAGE;
- (b) substrate specificity for primary and secondary alcohols and aldehydes;

Amendment Dated: November 10, 2009 Reply to Office Action Dated: May 11, 2009

- (c) pH-stability at pH of about 6 to about 9;
- (d) pH-optimum at pH of about 8.0; and
- (e) inhibited by Cu²⁺, Zn²⁺, Mn²⁺, Fe²⁺, and Fe³⁺.
- 11. (Withdrawn): A process according to claim 3 comprising (a) contacting the enzyme with the substrate and (b) isolating the L-gulono-1,4-lactone or L-gulonic acid from the reaction mixture.
- 12. (Withdrawn): A process according to claim 4 comprising (a) contacting the enzyme with the substrate and (b) isolating the L-galactono-1,4-lactone or L-galactonic acid from the reaction mixture.
- 13. (Previously presented): A process according to claim 2, wherein the process is conducted for 1 to 120 [[h]] hours at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.
- 14. (Withdrawn): A process according to claim 3, wherein the process is conducted for 1 to 120 h at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.
- 15. (Withdrawn): A process according to claim 4, wherein the process is conducted for 1 to 120 h at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.
- 16. (Previously presented): A process according to claim 8, wherein the process is conducted for 1 to 120 [[h]] hours at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.

Amendment Dated: November 10, 2009 Reply to Office Action Dated: May 11, 2009

17. (Withdrawn): A process according to claim 11, wherein the process is conducted for 1 to 120 h at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.

18. (Withdrawn): A process according to claim 12, wherein the process is conducted for 1 to 120 h at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.